Ethyl glucuronide in scalp and non-head hair: An intra-individual comparison

Pianta, A ; Liniger, B ; Baumgartner, M R

Abstract: AIMS: Analysis of ethyl glucuronide (EtG), a minor metabolite of ethanol, is a valid tool for the assessment of social and chronic excessive alcohol consumption. Standardized analysis of EtG is usually done in head hair. As head hair cannot always be provided, alternative hair matrices become more and more interesting. Therefore, a study was performed that compared the intra-individual EtG concentrations in scalp hair and non-head hair (chest, arm, leg and axillary hair). METHODS: Hair samples were collected from 68 subjects undergoing an expert assessment for fitness to drive. Aqueous extracts of the hair matrix were cleaned by solid-phase extraction, using an Oasis MAX column. EtG was first derivatized with perfluoropentanoic anhydride and then quantified by GC-MS/MS in negative chemical ionization mode, using EtG-d5 as internal standard. RESULTS: For categorizing drinking behaviour, the two EtG cut-off values recommended by the Society of Hair Testing were applied for all different hair types. For chest, arm and leg hair, correct classification ratios were >83%. This corresponds to sensitivity values >78% and specificities >75%. Such values indicate together with coefficients (r) > 0.7 a high correlation of the categorization of the drinking behaviour based on these body hair EtG concentrations compared with the indexing based on scalp hair EtG-values. However, it must be taken into consideration that the time frame represented by non-head hair may extend way back. CONCLUSIONS: These results indicate that chest, arm and leg hair can be a valid alternative to assess the drinking behaviour of a subject if head hair is not available; whereas axillary hair is not suitable as alternative matrix.

DOI: https://doi.org/10.1093/alcalc/agt012

Alcohol and Alcoholism Vol. 48, No. 3, pp. 295–302, 2013
Advance Access Publication 26 February 2013
doi: 10.1093/alcalc/agt012

ORIGINAL ARTICLE

Ethyl Glucuronide in Scalp and Non-head Hair: An Intra-individual Comparison

A. Pianta[1,*], B. Liniger[1] and M. R. Baumgartner[2]

[1]Department of Traffic Medicine and Forensic Psychiatry, Institute for Forensic Medicine, University of Zurich, Kurvenstrasse 31, Zurich 8006, Switzerland and [2]Center for Forensic Hairanalytics, Institute for Forensic Medicine, University of Zurich, Kurvenstrasse 17, Zurich 8006, Switzerland

*Corresponding author: Verkehrsmedizin & Forensische Psychiatrie, Institut für Rechtsmedizin, Kurvenstrasse 31, 8006 Zürich, Switzerland, Tel.: +41-43-259-56-90; Fax: +41-259-56-89; E-mail: alo.pianta@irm.uzh.ch

(*Received* 14 *August* 2012; *first review notified* 4 *October* 2012; *in revised form* 6 *December* 2012; *accepted* 24 *January* 2013)

Abstract — **Aims**: Analysis of ethyl glucuronide (EtG), a minor metabolite of ethanol, is a valid tool for the assessment of social and chronic excessive alcohol consumption. Standardized analysis of EtG is usually done in head hair. As head hair cannot always be provided, alternative hair matrices become more and more interesting. Therefore, a study was performed that compared the intra-individual EtG concentrations in scalp hair and non-head hair (chest, arm, leg and axillary hair). **Methods**: Hair samples were collected from 68 subjects undergoing an expert assessment for fitness to drive. Aqueous extracts of the hair matrix were cleaned by solid-phase extraction, using an Oasis MAX column. EtG was first derivatized with perfluoropentanoic anhydride and then quantified by GC-MS/MS in negative chemical ionization mode, using EtG-d5 as internal standard. **Results**: For categorizing drinking behaviour, the two EtG cut-off values recommended by the Society of Hair Testing were applied for all different hair types. For chest, arm and leg hair, correct classification ratios were >83%. This corresponds to sensitivity values >78% and specificities >75%. Such values indicate together with ϕ coefficients (r_ϕ) > 0.7 a high correlation of the categorization of the drinking behaviour based on these body hair EtG concentrations compared with the indexing based on scalp hair EtG-values. However, it must be taken into consideration that the time frame represented by non-head hair may extend way back. **Conclusions**: These results indicate that chest, arm and leg hair can be a valid alternative to assess the drinking behaviour of a subject if head hair is not available; whereas axillary hair is not suitable as alternative matrix.

INTRODUCTION

The quantitative determination of ethyl glucuronide (EtG) in scalp hair as a direct alcohol marker has become a standard method in both clinical and forensic toxicology (Wurst *et al*., 1999). It is a useful aid in the retrospective assessment of alcohol consumption, providing a specific window of detection that depends on the length of the hair sample (Liniger *et al*., 2010; Kharbouche *et al*., 2012).

In general, one unit of alcohol consists of 8–9 g of pure alcohol in an alcoholic beverage, equivalent to a glass (10 cl) of wine or one glass (25 cl) of beer. The consumption of > 4 units of alcohol per day for men and >2 units per day for women is considered harmful and, in the long term, has been shown to constitute a health risk. The World Health Organization guidelines (WHO, 2000) and the Swiss Institute for the Prevention of Alcohol and Drug Problems (Schweizerische Fachstelle für Alkohol- und andere Drogenprobleme, 2013) define risk categories on the basis of the amount of alcohol consumed. The three categories are abstinence, low-risk drinking and high-risk drinking. In adults, low-risk drinking is the daily consumption of <40 g pure ethanol for women and <60 g for men. Anything above those levels is considered high-risk drinking.

When assessing alcohol consumption by means of hair analysis, the EtG concentration is allocated to one of three categories: 'negative', 'social drinking' and 'chronic excessive consumption'. According to the recommendations of the Society of Hair Testing (SoHT, 2012), the threshold between 'social drinking' and 'chronic excessive alcohol consumption' is the higher cut-off of 30 pg/mg (Kintz, 2012). Values below the lower cut-off of 7 pg/mg are 'negative' and indicate a non-relevant alcohol consumption or abstinence (Swiss Society of Legal Medicine SGRM, 2009; Society of Hair Testing, 2012). If EtG is not detected at all, this may indicate total abstinence but the consumption of a small quantity of alcohol cannot be excluded. These threshold values are derived from different studies based on the analysis of scalp hair, which is the preferred specimen for EtG analysis (Skopp *et al*., 1995; Yegles *et al*., 2001; Wurst *et al*., 2003; Appenzeller *et al*., 2007; Pirro *et al*., 2011a; Kharbouche *et al*., 2012).

Body hair can be of interest in those cases where no scalp hair is available. So far, there have been only a few studies investigating the suitability of non-head hair for EtG monitoring. It could be shown in these studies that axillary and pubic hair are not suitable to assess drinking behaviour, whereas chest hair could be considered an alternative matrix for the determination of EtG in secondary hair (Kerekes *et al*., 2009; Pirro *et al*., 2011b). The incorporation mechanism of EtG into the hair matrix is still not fully known. There is evidence that the dominant route is incorporation in the hair root from blood (Schräder *et al*., 2012). However, for all different types of body and scalp hair a congeneric route of incorporation can be assumed.

For forensic purposes, it is of great importance to know the time frame within which the consumption behaviour was monitored. In general, this time period correlates with the hair length: the longer the hair, the wider the time frame. For the estimation of this time period, two main factors have to be taken into account. These are the hair growth rate during the anagen phase and the percentage of the hair in the telogen phase. Hair growth is a highly active metabolic process. The duration of the different phases of the hair growth cycle (anagen—catagen—telogen) depends on the type and localization of the hair follicle. Table 1 gives an overview of literature describing these two factors in scalp hair and body hair. It is obvious that these two parameters (growth rate and percentage of hair in telogen phase) differ significantly between scalp hair and hair from different body

Table 1. Summary of hair growth parameters: growth rate, percentage of hair in the anagen and telogen phases and estimated duration of these phases (month)

Hair type	Growth rate (cm/month)	Anagen phase (months)	Anagen phase (%)	Telogen phase (months)	Telogen phase (%)	Overall cycle time (months)	References
Scalp hair		48–144		2–6			Attenberger and Würfl (1981)
Scalp hair	1.05	6–18	85	3–4.25	13		Richards *et al.* (1990) and Richards and Meharg (1991/1997)
Scalp hair	0.6–1.5	24–144	80–85	6	10–15		Pragst *et al.* (1998)
Scalp hair	0.84–1.41	48–144			5–20		Pragst (2005)
Scalp hair	1.05	24–72		3			Vogt *et al.* (2008)
Scalp hair	1	60	85	5	10		[a]
Chest hair	1.05	4	30	4.5	70		Richards *et al.* (1990) and Richards and Meharg (1991/1997)
Chest hair	0.81				50	4–10	Pragst and Sachs (2007)
Chest hair	1	4	40	4.5	60		[a]
Axillary hair	0.9	4	30	3	70		Richards *et al.* (1990) and Richards and Meharg (1991/1997)
Axillary hair	0.87–0.99				50	23–35	Pragst and Sachs (2007)
Axillary hair	0.87–1.00	11–18			50		Pragst (2005)
Axillary hair	0.9						Vogt *et al.* (2008)
Axillary hair	0.9	9	40	3	60		[a]
Arm hair	0.9	3.25	20	3	80		Richards *et al.* (1990) and Richards and Meharg (1991/1997)
Arm hair		1–4			60		Pragst (2005)
Arm hair					60	4–6	Pragst and Sachs (2007)
Arm hair		1.5–3		1.75–3.25			Vogt *et al.* (2008)
Arm hair	0.9	3	30	3	65		[a]
Leg hair	0.63	4	20	6	80		Richards *et al.* (1990) and Richards and Meharg (1991/1997)
Leg hair	0.39–0.75					3–6	Pragst and Sachs (2007)
Leg hair	0.6	4.75–6.5		3.25–8.5			Vogt *et al.* (2008)
Leg hair	0.7	5	20	6	70		[a]

[a]Used in this study.

regions. This makes it difficult to find a common definition to estimate the time window for scalp and body hair, respectively.

The present study represents an intra-individual comparison of EtG in scalp hair and body hair. The aim of the study was to compare the categorization of the alcohol consumption behaviour based on EtG measured in different types of hair samples. Furthermore, the suitability of the established cut-off values for scalp hair was investigated for body hair.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were of analytical purity grade. EtG and the internal standard EtG-d5 were purchased by MediChem (Stuttgart, Germany); pentafluoropropionic anhydride (PFPA) and formic acid were obtained from Sigma-Aldrich (Buchs, Switzerland). Acetone, hexane, methanol, ethyl acetate (dried over molecular sieve (Union Carbide AW 300 4 Å, from Fluka (Switzerland)) and ammonia 25% were from Merck (Darmstadt, Germany).

Hair samples

Scalp and secondary hair samples were obtained from 68 subjects. The samples were provided as part of an expert assessment for fitness to drive, in order to confirm the alcohol consumption claimed. Scalp hair values were used in the assessment process. This corresponds to the standard procedure for forensic expert reports (Swiss Society of Legal Medicine SGRM, 2009). The investigations were performed on hair samples from volunteers after informed consent according the declaration of Helsinki (World Medical Association, 2008). The conditions for voluntary participation in the study were consistent drinking behaviour over at least the last 6 months, no cosmetic treatment of the hair such as tinting, dyeing, perming or bleaching, scalp hair of at least 2–4 cm in length and an adequate quantity of secondary hair. Two tufts of scalp hair were cut off as close to the head as possible. Secondary hair from the chest, arms, legs and/or axilla was shaved off with a disposable razor. The entire length of the secondary hair was analysed, while only the proximal segment of the scalp hair was tested up to a maximal length of 5 cm. Not all subjects could provide hair samples from all different body regions.

Drinking behaviour over the preceding 6 months was determined with the Alcohol Use Disorders Identification Test (AUDIT) questionnaire (Babor *et al.*, 1989). Question numbers 1 and 2 of the questionnaire allow an estimation of the average alcohol consumption per day to be calculated as ethanol daily intake (EDI, g/d). Additionally, the frequency of hair washing per week and the use of special shampoos were recorded.

Hair length and the window of detection

Hair of a given length correlates with a time frame defined by minimum and maximum values, respectively. The minimum level of this frame is determined by hair in the anagen phase. It can be calculated by the ratio of hair length to growth rate. On the other hand, for the estimation of the

maximum value, the entire hair growth cycle has to be taken into account. As a rule of thumb to estimate the maximum value of the time window the following formula is suggested in this study:

$$\text{Time window} = \frac{\text{Hair length}}{\text{Growth rate} \times ((1 - \text{Telogen\% })/100)}$$

For scalp hair with follicles persisting for one cycle for up to 6 years, with a mean growth rate of 1.1 cm/month and with a mean telogen percentage of 10%, the calculated maximum of the time window in months is equal to the measured length of the hair strand in cm. However, this estimation of the time frame is much more complicated for body hair as they exhibit a higher amount of hair in the telogen phase. Body hair of one subject does not have a uniform length but an asymmetrical length-distribution (Seago and Ebling, 1985). Therefore, for the calculation of the maximum value of the time frame for body hair, the 75% percentile was used together with the growth rate and percentage of telogen hair given in Table 1.

Extraction procedure and sample preparation

EtG was determined quantitatively by means of gas chromatography-negative ion chemical ionization–tandem mass spectrometry (GC–NICI/MS/MS) using the method first described by Yegles *et al*. (2001) and modified by Kerekes *et al*. (2009). Analysis and assessment of the results were in line with the recommendations of the Swiss Society of Legal Medicine SGRM (2009).

Between 20 and 30 mg of hair samples were washed twice (15 ml of deionised water followed by 10 ml of acetone for 3 min each) with vigorous shaking. After drying, the hair was finally cut and pulverized in a 2 ml Eppendorf tube with a tungsten carbide ball (7 mm diameter) on a bench top shaker. Extraction was done with 1.5 ml of deionized water and 50 µl of EtG-d5 as internal standard (IS, 20 pg/µl). After centrifugation, a solid-phase extraction was done using an Oasis MAX column, previously conditioned with 2 ml of methanol and 2 ml of deionized water. Once the sample was applied to the cartridge, the column was washed with 1 ml of water/ammonia (95/5, v/v) followed by 2 ml of methanol. After drying for 10 min, elution was achieved using 2 ml of methanol/formic acid (98/2, v/v). The eluate was dried under nitrogen at 35°C. The residue was derivatized with 80 µl PFPA, dried under nitrogen and reconstituted with 50 µl of ethyl acetate.

GC-MS/MS conditions

A Trace GC Ultra gas chromatograph coupled to a TSQ Quantum GC mass spectrometer (both from Thermo Scientific) was used for the quantification of EtG. The gas chromatograph was running with a programmed temperature Vaporization (PTV) injector in back-flush mode. Additionally, the system was equipped with a TriPlus autosampler (Thermo). Chromatographic separation was achieved using a fused silica capillary column J&W DB-5 MS (30 m length × 0.25 mm i.d. × 0.25 µm film thickness) coupled to a T-connector to a precolumn (J&W DB-5 MS, 1 m length × 0.53 mm i.d. × 0.50 µm film thickness). The carrier gas was helium at a constant flow of 2 ml/min. One microlitre was injected in splitless mode on the PTV injector at 250°C. The transfer line was held at 200°C. The initial oven temperature of 80°C was kept for 3 min, increased first at 25°C/min up to 200°C and then ramped at 40°C/min up to 280°C. Samples were ionized by negative chemical ionization with methane as reagent gas (flow 2.0 ml/min). The ion source temperature was kept at 140°C with an emission current of 150 µA. Data acquisition was performed in the selected reaction mode. The collision energy was constant at 7 eV for the transitions *m/z* 496 163 and *m/z* 501 163 (quantifier), respectively, and transitions *m/z* 377 163 and *m/z* 382 163 and transitions *m/z* 349 119 and *m/z* 354 119 (qualifiers). The system was run and the data were evaluated with the Excalibur software 1.5.2.

Categorization of drinking behaviour and statistical evaluation

For each type of body hair, a Wilcoxon test was used for an overall comparison of EtG concentration in scalp hair and the one determined in the corresponding body hair samples of each individual.

Based on the EtG concentrations, scalp and secondary hair samples were allocated to one of three drinking categories: 'negative' (i.e. non-relevant alcohol consumption or abstinence), 'social drinking' (also referred to as moderate consumption) or 'chronic excessive consumption' (also long-term heavy drinking), using two cut-off values. The lower cut-off of 7 pg/mg separated 'social drinking' from 'negative', while the higher cut-off of 30 pg/mg was used to distinguish 'chronic excessive consumption' from 'social drinking'. The significance of this categorization was evaluated by Fisher's exact test, because the number of cases was too small for an examination with a χ^2-test.

After categorizing all hair samples, the indexing of each secondary hair was compared with the indexing of the corresponding scalp hair as reference. Equal classification of body hair sample with the corresponding scalp hair sample was evaluated as 'true', below the cut-off as 'true negative' (TN) and above the cut-off as 'true positive' (TP). Unequal classifications were evaluated as 'false positive' (FP) and as 'false negative' (FN), respectively. Based on these four frequencies by category of a 2 × 2 contingency table, the sensitivity (Sens.), the specificity (Spec.), the correct classification rate (CCR) and the ϕ coefficient r_ϕ were calculated to evaluate the significance of the categorization of the drinking behaviour based on EtG concentrations determined in different body hair samples. This analysis was done separately for both threshold limits (lower cut-off 7 pg/mg and higher cut-off 30 pg/mg) and for each type of body hair.

$$\text{Sens.} = \frac{\text{TP}}{\text{TP+FN}}$$

$$\text{Spec.} = \frac{\text{TN}}{\text{TN+FP}}$$

$$\text{CCR} = \frac{\text{TP+TN}}{\text{TP+FN+ TN+FP}}$$

$$r_\varphi = \frac{(\text{TP} \times \text{TN}) + (\text{FP} \times \text{FN})}{\sqrt{(\text{TN+FN})(\text{TP} + \text{FP})(\text{TN} + \text{FP})(\text{TP+FN})}}$$

RESULTS AND DISCUSSION

In total, hair samples from 68 subjects were analysed in this study. Scalp hair was obtained from all 68 volunteers aged from 21 to 68 years (mean 38.6 years). The gender distribution was 98.5% ($n = 67$) male and 1.5% ($n = 1$) female. Women commonly lack secondary hair often simply because of cosmetic treatment. Additionally, females are underrepresented in our collective of people undergoing an assessment for fitness to drive (13.3%). Overall, hair samples from all body regions could not be provided in all cases. 61 leg hair samples, 51 arm hair samples, 48 chest hair samples and 10 axillary hair samples could be obtained. Age, frequency of hair washing, EDI values, EtG concentrations and experimentally determined hair lengths of all cases are summarized in Table 2.

EtG concentrations in scalp hair and secondary hair

EtG concentrations ranged from not detectable (ND) up to 1600 pg/mg (mean 79 pg/mg, median 23 pg/mg) in scalp hair, from ND up to 520 pg/mg (mean 63 pg/mg, median 24 pg/mg) in chest hair, from ND up to 880 pg/mg (mean 87 pg/mg, median 43 pg/mg) in arm hair, from ND up to 970 pg/mg (mean 84 pg/mg, median 30 pg/mg) in leg hair and from ND up to 20 pg/mg (mean 6 pg/mg, median 4 pg/mg) in axillary hair. Scalp and chest hair had approximately the same median value. The corresponding values for leg hair and arm hair were slightly higher than for scalp hair, whereas the EtG-median value in axillary hair was around six times lower (Fig. 1).

Because not all of the 68 subjects could provide all types of body hair samples, the comparison between scalp and body hair was performed only with paired samples. Thus, the *P*-values calculated with the Wilcoxon test revealed no significant difference in the EtG concentrations for the paired groups scalp and chest hair (*P*-value 0.419), scalp and arm hair (*P*-value 0.259) and scalp and leg hair (*P*-value 0.131), respectively. In contrast, the distribution of EtG concentrations in axillary hair differed significantly from that in scalp hair (*P*-value 0.005). This result corresponds to findings from prior studies (Kerekes *et al*., 2009; Pirro *et al*., 2011b). These significantly lower concentrations observed in axillary hair can probably be explained by a decomposition of EtG by chemical ingredients of deodorants. An additional explanation might be an increased leaching process due to sweat.

Hair length and the window of detection

The distribution of the hair lengths and the calculated time frames are shown in Figure 2. The calculated maximum values correlate with the overall hair growth cycle time estimated from literature data: chest hair 9.1 months (calculated; literature data 8.5 and 10 months (Richards *et al*., 1990; Richards and Meharg, 1991/1997; Pragst and Sachs, 2007)), arm hair 6.3 months (calculated; literature data 6.25 and 7 months (Richards *et al*., 1990; Richards and Meharg, 1991/1997; Pragst, 2005; Pragst and Sachs, 2007; Vogt *et al*., 2008)) and leg hair 11.9 months (calculated; literature data 6, 10 and 15 months (Richards *et al*., 1990; Richards and Meharg, 1991/1997; Pragst and Sachs, 2007; Vogt *et al*., 2008)), respectively.

The calculated time windows for arm hair are closest to those represented by scalp hair. The windows of detection extended furthest back for leg hair and were sometimes considerably more than the self-reported 6 months of consistent alcohol consumption declared for study purposes.

Intra-individual comparison of drinking category

The categories of drinking behaviour are separated by the two cut-off values 7 and 30 pg/mg for the EtG concentrations. These values are established for scalp hair. Therefore, the indexing of the scalp hair into the three categories ('negative', 'social drinking' and 'chronic excessive alcohol consumption') was taken as reference. Categorization of body hair samples using the same cut-off values was compared intra-individually with this reference.

Category 'negative'

Eleven cases were classified as 'negative' (EtG < 7 pg/mg) based on the EtG concentration in scalp hair. There was 100% match with chest hair samples ($n = 7$). Arm hair samples matched in 75% ($n = 6$ of 8) of cases and leg hair samples were assigned to the same category in 91% ($n = 10$ of 11) as shown in Figure 3C. EtG concentrations from two of the three body hair samples assigned to a higher category (Case 19, arm and leg hair, Table 2) were equal to the lower cut-off value.

Category 'social drinking'

Considering the EtG concentrations in scalp hair ($n = 27$) rated as 'social drinking', intra-individual comparison showed that 17–24% of the secondary hair samples were in the higher consumption category (chest, arm and leg hair) and 12–25% in the lower category (Fig. 3B). Half of the 12 body hair samples classified in the higher category originated from three cases with scalp hair EtG-values of 15, 19 and 28 pg/mg, respectively. Six of the ten body hair samples categorized in the lower class originated again from three cases with EtG-values close to the cut-off of 7 pg/ml (scalp hair concentrations: 8 and 10 pg/mg, respectively).

For axillary hair, a completely different situation was observed. Five (83%) of these six samples were in the lower category and in three cases the EtG concentrations in axillary hair were even below the limit of detection. Axillary hair had lower EtG concentrations (Fig. 1) and tended to be classified lower but never higher than scalp hair (Fig. 3).

Category 'chronic excessive drinking'

There was a good correlation, of 83–100%, between the categorization of scalp hair ($n = 30$) in the 'chronic excessive alcohol consumption' category and chest, arm and leg hair, as can be seen in Figure 3A. Again axillary hair showed much lower values in this category.

Statistical analysis

For the evaluation of the significance of the categorization of the drinking behaviour, the four values TP, TN, FP and FN of the 2×2 contingency table were used (Table 3). This analysis was done for each type of body hair separately and for

Table 2. All cases including age, hair washing habits, self-declared EDI, EtG concentrations in scalp and secondary hair and lengths of hair samples

Case	Age (years)	Wash (1 = daily; 2 = not daily)	EDI (g/d)	EtG scalp hair (pg/mg)	Length scalp hair (cm)	EtG chest hair (pg/mg)	Length chest hair (cm)	EtG arm hair (pg/mg)	Length arm hair (cm)	EtG leg hair (pg/mg)	Length leg hair (cm)	EtG axillary hair (pg/mg)	Length axillary hair (cm)
1	27	1	30.0	90	3.5			74	1.5	50	3.5		
2	34	1	18.2	14	4.5	20	1.0	8	3.5	10	3.0		
3	25	1	2.6	12	3.5			7	1.5	27	2.5		
4	23	2	8.0	34	2.5			34	2.5	50	2.0		
5	32	1	25.7	16	4.0	28	3.0						
6	46	2	9.7	18	4.0	17	4.0			26	2.0		
7	58	1	3.7	24	4.0	14	3.0	10	3.0	10	2.5	3	4.5
8	50	2	6.4	11	4.0	8	4.0			110	2.5		
9	42	1	4.2	36	4.0	30	4.0						
10	53	1	17.2	54	3.0	40	3.0	47	2.0	61	2.5		
11	25	1	10.6	100	3.0			130	1.5	56	2.5		
12	21	1	5.1	8	5.0			6	1.5	4	2.0		
13	21	1	34.2	99	3.0	120	2.0	72	2.0	56	2.0		
14	36	1	18.0	210	3.0	93	2.0	240	1.0	970	1.0		
15	45	2	3.2	28	2.0	16	2.0						
16	36	1	30.0	120	3.0	52	2.0	880	1.0	630	2.0		
17	25	1	7.7	63	4.5			64	1.5	34	2.5		
18	48	1	3.7	120	2.0	130	3.0	170	2.0				
19	28	1	0.5	1	4.0	4	3.0	7	1.0	7	2.0		
20	29	1	9.6	21	2.0	11	1.0			22	1.5	0	1.0
21	28	2	1.4	4	4.0					4	1.5	0	2.0
22	44	1	25.0	21	4.0	55	2.5			30	2.0		
23	28	1	15.3	16	4.0					24	2.0	10	2.5
24	24	1	7.0	8	4.0					9	2.0	1	3.0
25	39	1	11.4	88	4.0	77	5.0	69	2.0	49	2.0		
26	31	2	60.0	68	4.5			52	1.5	53	2.0	7	1.5
27	50	1	1.7	53	4.0	91	3.5	54	2.5	63	2.0		
28	52	1	1.9	26	4.0	60	2.5			46	2.5		
29	41	1	60.0	500	3.5	290	6.0						
30	33	1	1.2	3	4.0			4	2.0	3.0	2.5		
31	25	1	10.9	22	4.0			21	1.0	75.0	2.5	5	2.5
32	27	1	7.8	84	4.0			60	1.5	83.0	2.5	18	3.0
33	48	1	19.3	30	3.5			45	1.5	27.0	2.0		
34	21	1	1.7	10	2.5			5	1.5	10.0	2.0	0	1.5
35	57	2	32.0	100	4.0	110	2.0	150	1.0				
36	48	1	120.0	430	4.0	89	5.0						
37	63	2	32.1	43	4.0	93	3.0	220	2.0	470	2.0	20	4.5
38	32	2	1.0	4	4.0	2	4.0			4	2.0		
39	35	1	5.3	5	5.0			3	1.5	5	2.0		
40	31	2	11.0	1600	4.0	520	2.0	450	1.5	720	2.0		
41	57	1	50.0	210	3.0	340	3.5	350	1.5	290	2.0		
42	24	2	24.0	50	3.0	88	1.0	53	1.5	48	2.5		
43	47	1	17.1	19	5.0	39	3.5	65	2.0	29	3.0		
44	43	1	15.5	4	4.0	4	3.0	3	2.0	6	3.0		
45	25	1	21.4	54	4.0	7	3.0	40	2.0	14	3.0		
46	28	1	3.4	16	3.5	6	3.0	14	2.0	15	2.0		
47	27	2	2.5	10	4.0	6	2.0	6	1.5	6	0.5		
48	57	1	5.3	11	5.0	20	3.0	21	1.5	55	1.5		
49	42	2	4.3	3	4.0					3	2.0		
50	52	2	1.1	14	4.0	11	2.0			14	1.5		
51	53	2	30.0	19	4.0					37	1.5		
52	30	1	61.0	50	4.0			88	2.0	70	2.0		
53	24	1	40.0	15	4.0			42	2.0	48	2.0		
54	53	1	5.7	2	2.0	3	3.5	0	2.0	2	2.0		
55	43	1	5.7	8	4.0	10	2.0	10	1.5	24	2.0		
56	68	2	12.8	50	4.0	70	3.0	110	2.0	120	3.0		
57	39	1	40.0	110	4.0	68	4.5	43	2.0	54	3.0		
58	30	1	17.1	8	4.0	0	2.0	6	1.0	6	1.5		
59	49	1	5.4	5	4.0	3	4.0	11	3.0	6	3.0		
60	33	2	11.4	12	4.0	16	1.0	5	2.0	8	2.0		
61	34	1	0.3	0	4.0	0	2.0	0	1.0	0	1.0		
62	40	1	17.1	20	4.0	6	4.5	11	2.0	8	3.0		
63	37	2	0.6	0	3.0	3	2.5	0	2.0	0	2.5		
64	36	2	4.0	130	3.0	20	1.5	210	1.0	36	2.5		
65	54	1	12.6	34	4.0	30	4.0	55	2.0	42	2.5		
66	42	1	5.8	59	4.0	54	3.0	90	2.0	86	3.0		
67	50	2	25.7	54	3.5	19	2.0	36	2.0	80	2.5		
68	46	2	8.0	180	4.0	240	4.0	280	1.8	240	2.0		

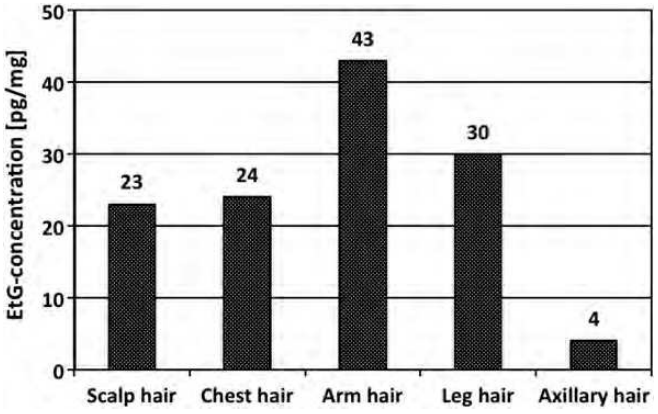

Fig. 1. Median values of all EtG concentrations (pg/mg) according to the type of body hair.

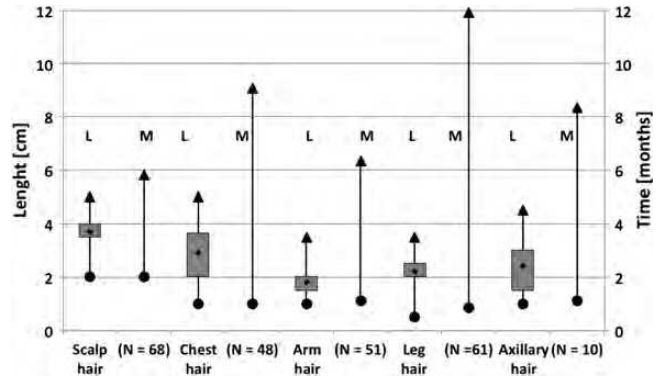

Fig. 2. Boxplot (including mean value) of the measured hair length (L, cm) of the scalp and body hair samples and the calculated time frame (minimum and maximum) corresponding to these hair samples (M, months). For calculation details see text.

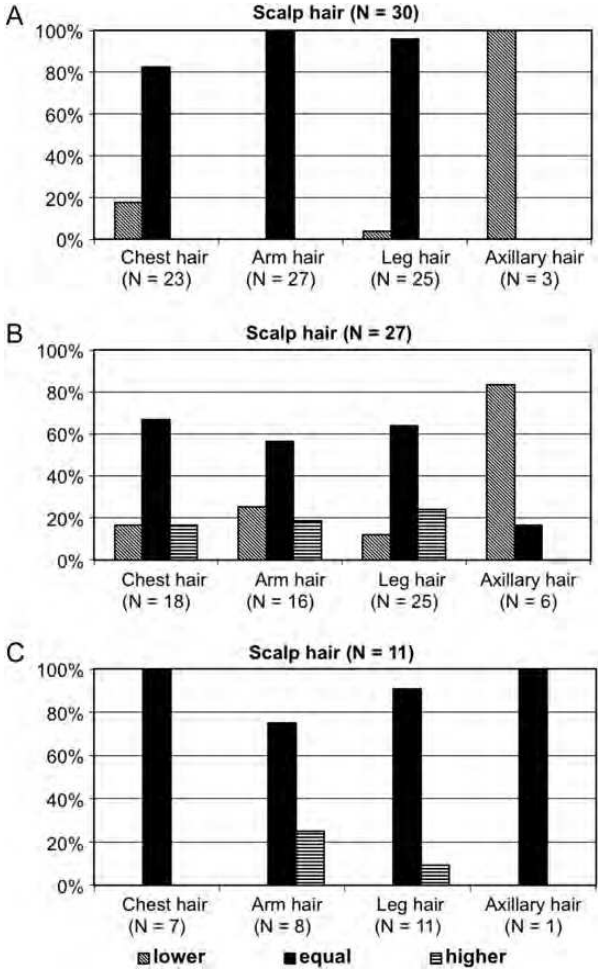

Fig. 3. Intra-individual comparison of the alcohol consumption category derived from scalp hair EtG concentration and the categorization based on the EtG-values observed in the different body hair samples: (A) category 'chronic excessive consumption', (B) category 'social drinking' and (C) category 'negative'. Lower and higher means that the classification based on body hair sample allocates to the next lower or higher category of drinking behaviour.

both threshold limits (lower cut-off 7 pg/mg and higher cut-off 30 pg/mg). The frequency distributions within these 2×2 contingency tables were highly significant ($P \leq 0.001$) except for axillary hair.

For chest, arm and leg hair CCRs were >83%. This corresponds to sensitivity values >78% and specificities higher 75%. Such values indicate together with coefficients (r_ϕ) >0.7 a high correlation of the categorization of the drinking behaviour based on body hair EtG concentrations compared with the indexing based on scalp hair EtG-values. Looking at the two cut-off values separately, it appears that for the lower one (7 pg/mg), chest hair and leg hair exhibit CCR-values of 92 and 93%, accompanied by an r_ϕ >0.7. For the higher cut-off (30 pg/mg), arm hair shows high values for CCR (94%) and r_ϕ (0.887), respectively.

Axillary hair with EtG concentrations consistently lower than scalp hair showed poor sensitivity, but a specificity of 100%. Additionally, low values result for r_ϕ and the CCR. Previous studies had shown that axillary hair is not suitable to assess drinking behaviour (Kerekes *et al.*, 2009; Pirro *et al.*, 2011b). These findings were confirmed by the present study. Decomposition of EtG in axillary hair is caused probably by chemical ingredients of deodorants. Leaching of EtG from the hair matrix by sweat can also result in decreased concentrations.

EDI values

EDI values ranged from 0.3 to 120 g alcohol per day. The mean was 16.5 g alcohol (median 10.2 g/d), which is equal to 2 units per day. The range of self-reported EDI values found in the 'negative' category of scalp hair ranged from 0.3 up to 15.5 g ethanol per day. In the 'social drinking' category of scalp hair, EDI values extended to 40 g ethanol per day, which is according to the World Health Organization guidelines (WHO, 2000) 'low-risk drinking' as well. When scalp hair was classified in the 'chronic excessive alcohol consumption' category, EtG concentrations tended to be higher than expected from the self-reported alcohol consumption. In the present study, 27 out of 68 cases showed EDI values <60 g/d, while EtG in head hair was >30 pg/mg.

Table 3. Sens., Spec., the CCR and coefficient (r_ϕ) calculated from the values TP, FP, TN, FN as well as the *P*-value (Fisher's exact test) of the categorization of the drinking behaviour based on EtG concentrations determined in secondary hair compared with scalp hair regarding the two threshold levels (A: lower cut-off value 7 pg/mg and B: higher cut-off 30 pg/mg). The cut-off values separate the 'negative' from the 'social drinking' and the 'social drinking' from the 'chronic excessive consumption' category

	n	TP	FN	TN	FP	Sens.	Spec.	*P*	r_ϕ	CCR
A: cut-off 7 pg/mg										
Chest hair	48	37	4	7	0	90%	100%	<0.001	0.758	92%
Arm hair	51	38	5	6	2	88%	75%	0.001	0.560	86%
Leg hair	61	47	3	10	1	94%	91%	<0.001	0.797	93%
Axillary hair	10	4	5	1	0	44%	100%	[a]	0.272	50%
B: cut-off 30 pg/mg										
Chest hair	48	18	5	22	3	78%	88%	<0.001	0.667	83%
Arm hair	51	27	0	21	3	100%	88%	<0.001	0.887	94%
Leg hair	61	24	1	30	6	96%	83%	<0.001	0.780	89%
Axillary hair	10	0	3	7	0	0%	100%	[a]	0	70%

[a]Not calculated, calculation assumption not fulfilled.

The EDI resulting from self-reported alcohol intake is a subjective parameter. A retrospective statement over the last 6 months and the situation of an assessment can possibly result in an under- or overestimation of one's alcohol consumption. Only in prospective studies including a daily alcohol self-monitoring log over several months, the EDI and the EtG findings correlate well (Kharbouche *et al*., 2012). This explains why 27 out 68 cases had a social drinking behaviour based on EDI while EtG in head hair was classified as 'chronic excessive alcohol consumption'.

Frequency of hair washing

The habit for hair washing might be different for scalp hair and body hair. EtG concentration in hair can be reduced by hair washing (Morini *et al*., 2010). The hair washing behaviour was allocated into two groups. Forty-seven (69%) subjects washed their scalp hair every day, 20 (29%) subjects washed it two or three times a week and one participant just once a week. With the exception of chest hair, the mean and median EtG concentrations were always lower in the group washing their hair on a daily basis. The decrease of the mean concentration for head, arm and axillary hair was between 40 and 50% and for chest and leg hair between 30 and 27%, respectively (Fig. 4).

One participant (Case 28) disclosed the daily use of a special strongly degreasing hair shampoo (name and brand not specified). The category of consumption in this case was for scalp hair 'social drinking' and for chest and leg hair 'chronic excessive consumption'. These findings may be attributed to an EtG washout phenomenon due to the degreasing action of the shampoo on scalp hair. If the incorporation of EtG in hair results also from sweat, the removal of EtG by a degreasing shampoo could be a possible explanation. This can lead to a lower assessment of consumption category in scalp hair.

Some extremely high EtG concentrations were found in five cases (Case 14, 16, 29, 36 and 40, see Table 2). These values were all above the cut-off value of 30 pg/mg and showed intra-individual variation of several 100 pg/mg. No particular features were noted with respect to hair length or washing habits in these five cases, so it remained unclear what caused these high EtG concentrations. As the EtG concentrations of all hair samples in these cases were clearly in the 'chronic excessive consumption' range, the large concentration differences do not affect their classification.

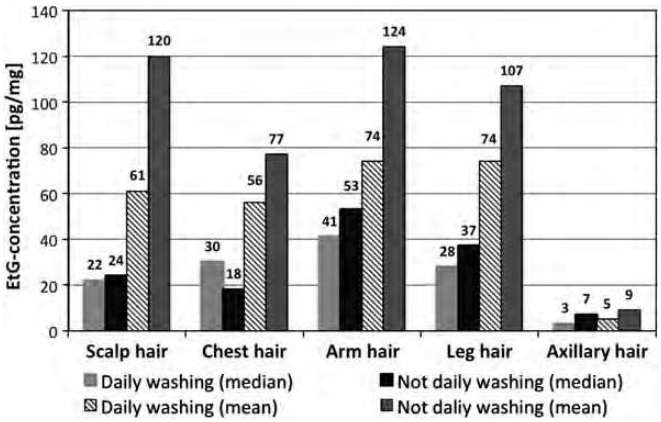

Fig. 4. Median and mean EtG concentrations (pg/mg) of scalp and body hair in the two groups hair washing 'daily' and 'not daily'.

CONCLUSIONS

Determination of the EtG concentration in hair samples from chest, arms and legs represents a useful alternative in cases where scalp hair cannot be sampled or analysed.

When using body hair, it must be taken into consideration that the time frame represented by these hair samples may extend way back because of the long telogen phases and the high percentage of hair in this phase. As a consequence, drinking behaviour in the recent past is only poorly documented and cannot be investigated separately by segmentation—in contrast to head hair.

Chest hair is to be preferred as the alternative to scalp hair for assessing alcohol consumption or abstinence. This applies particularly to abstinence monitoring (specificity 100%) with prolonged teetotalism of >6 months. In cases when chronic excessive consumption has been confirmed or heavy drinking is well documented in the past, chest hair, arm hair or leg hair are equally suitable.

It could be shown by this intra-individual comparison that the cut-off values established for scalp hair are also suitable for these types of body hairs.

Axillary hair is not suitable as alternative matrix to monitor drinking behaviour. Frequent washing and the use of strongly degreasing shampoos and chemicals may lead to a decrease in the EtG concentration of any hair samples. However, a limitation of this study is the small sample number of participants and an unbalanced gender distribution.

Acknowledgements — The authors thank their colleagues Dr Tina Binz and Oliver Scheidegger for their assistance and helpful discussions, Dr Martina Menn for the statistical calculations and all the employees who participated in the study.

Funding — There was no funding for this study. The costs for analysis of body hair were taken by our Institute of Forensic Medicine, Zurich.

Conflict of interest statement. None declared.

REFERENCES

Appenzeller BM, Agirman R, Neuberg P *et al*. (2007) Segmental determination of ethyl glucuronide in hair: a Pilot Study. *Forensic Sci Int* **173**:87–92.

Attenberger A, Würfl O. (1981) *Fachkunde für Friseure. 3. überarbeitete Auflage*. Augsburg: Ernst Kieser GmbH.

Babor TF, de la Fuente JR, Saunders J *et al*. (1989) *AUDIT The Alcohol Use Disorders Identification Test: Guidelines for Use in Primary Health Care. WHO/MNH/DAT/89.4*. Geneva, Switzerland: World Health Organization.

Kerekes I, Yegles M, Grimm U *et al*. (2009) Ethyl glucuronide determination: head hair versus non-head hair. *Alcohol Alcohol* **44**:62–6.

Kharbouche H, Faouzi M, Sanchez N *et al*.. (2012) Diagnostic performance of ethyl glucuronide in hair for the investigation of alcohol drinking behavior: a comparison with traditional biomarkers. *Int J Legal Med* **126**:243–50.

Kintz P. (2012) Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption 2011. *Forensic Sci Int* **218**:2.

Liniger B, Nguyen A, Friederich-Koch A *et al*. (2010) Abstinence monitoring of suspected drinking drivers: ethyl glucuronide in hair versus CDT. *Traffic Inj Prev* **11**:123–6.

Morini L, Zucchella A, Polettini A *et al*. (2010) Effect of bleaching on ethyl glucuronide in hair: an in vitro experiment. *Forensic Sci Int* **198**:23–7.

Pirro V, Valente V, Oliveri P *et al*. (2011a) Chemometric evaluation of nine alcohol biomarkers in a large population of clinically-classified subjects: pre-eminence of ethyl glucuronide concentration in hair for confirmatory classification. *Anal Bioanal Chem* **401**:2153–64.

Pirro V, Di Corcia D, Pellegrino S *et al*. (2011b) A study of distribution of ethyl glucuronide in different keratin matrices. *Forensic Sci Int* **210**:271–7.

Pragst F. (2005) Pitfalls in hair analysis. *TIAFT Bull* **35**:10–17.

Pragst F, Sachs H. (2007) Die Haarprobe als Untersuchungsmatrix zur toxikologischen Fahreignungsdiagnostik. Satelliten Symposium "Toxikologische Aspekte der Fahreignung". Tagungsband zum XV. GTFCh-Symposium 2007. Mosbach/Baden, p. 84–99.

Pragst F, Rothe M, Spiegel K *et al*. (1998) Illegal and therapeutic drug concentrations in hair segments—a time table of drug exposure? *Forensic Sci Rev* **10**:81–111.

Richards RN, Meharg GE. (1991/1997) *Cosmetic and Medical Electrolysis and Temporary Hair Removal: A Practice Manual and Reference Guide*. 2nd edn. Toronto: Medric Ltd.

Richards RN, Uy M, Meharg GE. (1990) Temporary hair removal in patients with hirsutismus: a Clinical Study. *Cutis* **45**:199–202.

Schräder J, Rothe M, Pragst F. (2012) Ethyl glucuronide concentrations in beard hair after sinlge alcohol dose: evidence for incorporation in hair root. *Int J Legal Med* **126**:791–9.

Schweizerische Fachstelle für Alkohol- und andere Drogenprobleme (2013) http://www.suchtschweiz.ch/infos-und-fakten/alkohol/konsum/risikokonsum/ (10 August 2012, date last accessed).

Seago SV, Ebling FJG. (1985) The hair cycle on the human thigh and upper arm. *Br J Dermatol* **113**:9–16.

Skopp G, Schmitt G, Drönner P *et al*. (1995) Trinkverhalten und Haaranalyse. GTFCh-Symposium Arzneistoffmißbrauch. Heppenheim, Verlag: Dr. Dieter Helm. p. 175–9.

Society of Hair Testing. (2012) Use of alcohol markers for abstinence assessment 2012. Consensus of the Society of Hair Testing. http://www.soht.org/pdf/Use of Alcohol Markers in Hair for Abstinence Assessment 2012.pdf (10 August 2012, date last accessed).

Swiss Society of Legal Medicine SGRM, Working Group on Hair Testing. (2009) "*Die forensisch-toxikologische Haaranalytik" bzw.* "*Bestimmung von EtG in Haarproben"*. http://www.sgrm.ch (10 August 2012, date last accessed).

Vogt A, McElwee KJ, Blume-Peytavi U. (2008) Biology of the hair follicle. In Blume-Peytavi U, Tosti A, Whiting DA, Trüeb RM (eds), *Hair Growth and Disorders*. Berlin, Heidelberg: Springer-Verlag, p. 13.

World Health Organization (2000) International guide for monitoring alcohol consumption and related harm. WHO/MSD/MSB/00.4:52–4.

World Medical Association (2008) Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. In: Adopted by the 18th WMA General assembly, Helsinki, Finland, June 1964, and amended last by the 59th WMA General Assembly. Seoul. http://www.wma.net/en/30publications/10policies/b3/17c.pdf (10 August 2012, date last accessed).

Wurst FM, Kempter C, Seidl S *et al*. (1999) Ethyl glucuronide—a marker of alcohol consumption and a relapse marker with clinical and Forensic implication. *Alcohol Alcohol* **34**:71–7.

Wurst FM, Skipper GE, Weinmann W. (2003) Ethyl glucuronide—the direct ethanol metabolite on the threshold from science to routine use. *Addiction* **98**:51–61.

Yegles M, Panarotto E, Labarthe A *et al*. (2001) Determination by GC-MS/NCI of EtG in Hair. In: Beiträge zum XII. Symposium der GFTCh. Heppenheim, Verlag: Dr. Dieter Helm, p. 299–303.